Acute Nitric Oxide Blockade Amplifies the Renal Vasoconstrictor Actions of Angiotension II[1,2]

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ABSTRACT

The tone in the renal vasculature is determined by the balance between vasoconstrictor and vasodilator agents. In this study, the effect on renal function was investigated when the acute blockade of the endogenous nitric oxide system was superimposed on a state of high circulating angiotensin II. Studies were conducted in the conscious, unstressed rat measuring renal function before and during acute systemic nitric oxide blockade with nitro-L-arginine methyl ester and with or without concomitant angiotensin II infusion. Nitric oxide blockade alone, in the presence of normal, unstimulated levels of endogenous angiotensin II, caused a large rise in blood pressure and a doubling of renal vascular resistance. The infusion of angiotensin II alone produced a mild rise in systemic blood pressure and a small (30%) rise in renal vascular resistance. When nitric oxide blockade was combined with angiotensin II infusion, the rise in blood pressure was similar to that produced by nitric oxide blockade alone but the increase in renal vascular resistance was much greater (350%), leading to marked declines in renal function. These studies demonstrate that when angiotensin II levels are acutely elevated and are controlling renal vascular tone, nitric oxide is essential for the maintenance of adequate renal perfusion and function.

Key Words: *Nitroarginine methyl ester, GFR, RPF, sodium excretion, conscious rat*

Acute blockade of nitric oxide (NO) production in the rat produces a large rise in blood pressure (BP) and renal vascular resistance (RVR) (1–3). In the conscious, unstressed preparation, we have reported that the blockade of endogenous angiotensin II (AII) does not ameliorate the pressor or renal vasoconstrictor actions of acute NO blockade (4), suggesting that AII does not contribute to this vasoconstrictor response. In contrast, in the anesthetized rat subjected to acute surgery, some workers have reported that AII blockade blunts the renal but not the systemic vasoconstrictor response to NO blockade (2,3), suggesting that AII does contribute to this renal vasoconstrictor response. Sigmon and Beierwaltes have shown that the AII dependence of NO blockade–induced renal vasoconstriction varies according to the preparation and is much less pronounced in the normal, awake animal in whom the AII system is not activated (5). These studies were conducted to examine the response of the renal vasculature to NO blockade in the conscious rat when circulating AII levels had been raised by infusion.

METHODS

Studies were conducted on 21 male Sprague-Dawley rats (obtained from Harlan Sprague Dawley Inc.) (aged 4 to 5 months) maintained on a diet containing 24% protein and 0.5% sodium. In preliminary surgery, catheters were placed in the left femoral artery and vein and in the urinary bladder. The vascular catheters were exteriorized at the back of the neck, primed with a 1:1 solution of dextrose (50%) and heparin (1,000 U/mL), and plugged. The bladder was irrigated with a neomycin solution and plugged so that rats were able to void normally through the urethra. This surgery was conducted under full sterile conditions and with the rats under barbiturate-induced general anesthesia. Further details of this preparation are given elsewhere (1,4).

Seven days after surgery, renal function experiments were conducted as follows. Rats were placed in a restraining cage, the bladder pin was removed for the collection of urine, and the indwelling arterial catheter was used for the recording of BP and the occasional sampling of arterial blood. A continual iv infusion was given of a 0.9% NaCl solution containing tritiated inulin (2 to 5 μC/mL) and *p*-aminohippurate (1%), at the rate of 5 μL/min per 100 g rat body wt. After 80 min, when plasma inulin and PAH concentrations had reached a plateau, a control observation period was begun in which two 20- to 30-

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min urine collections were made and arterial blood samples (150 μL) were taken at the midpoint of each urine collection.

At the end of control measurements, one of the following three protocols was carried out. In Group 1 ($N = 7$), rats received an iv bolus of 10 mg/kg of the NO synthesis inhibitor N^G-nitro-L-arginine methylester (NAME). In Group 3 ($N = 6$), rats received the bolus NAME and a continual iv infusion of AII was also given in a dose of 5 ng/kg per minute (AII concentration, 100 ng/mL in the inulin/PAH-containing infusate). Ten minutes after drug administration, two 20-min urine collections with midpoint blood samplings were taken. Group 2 ($N = 8$) received the AII infusion alone and were studied for a single period; the data for this group have been published previously (6).

At the end of all experiments, red blood cells removed during blood sampling were reconstituted with sterile isotonic NaCl and restored to the rat. Rats were returned to their home cages and were later euthanized with an overdose of barbiturate; the bladder and kidneys were examined and found to be free of infection.

The volume of all urine samples was measured gravimetrically, and the urine was analyzed for tritiated inulin activity and PAH and sodium concentrations. Arterial blood samples were analyzed for hematocrit, tritiated inulin activity, and concentrations of PAH and sodium. Details of these analyses have been published previously (1,4). These measurements allow the calculation of inulin clearance (= GFR); PAH clearance factored for renal PAH extraction (= RPF); RVR; urinary excretion of sodium and potassium; and the fractional excretion of sodium. These calculations are described elsewhere (7). Data are expressed throughout as mean ± SE. Within-group statistical significance ($P < 0.05$) was determined by paired t test. Comparisons on absolute data between groups were by two-way analysis of variance (ANOVA). Comparisons on percent change from control, between groups, were by one-way ANOVA.

All animal experiments described in this article were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with the approval of the West Virginia University Animal Care and Use Committee.

RESULTS

As shown in Table 1, the acute systemic blockade of NO in Group 1 rats produced large rises in BP and RVR, with a fall in RPF and a lesser reduction in GFR; thus, filtration fraction (FF) increased. Natriuretic and diuretic responses also occurred, and the fractional excretion of sodium (FE_{Na}) rose. These findings are similar to those reported earlier by us in the conscious rat, and as shown previously, the dose of NAME used in these studies (10 mg/kg) produces the maximum increment of BP (1). Group 2 rats received AII alone, which produced a mild pressor and renal vasoconstrictor response, with a small fall in RPF, no change in GFR, a rise in FF, and no change in Na excretion ($U_{Na}V$) or urine flow (V) (Table 1). As shown in Figure 1, the magnitude of the rise in RVR and BP was less than that seen in Group 1 rats receiving NO blockade alone. Group 3 rats received the same dose of NAME, producing systemic NO

TABLE 1. Summary of BP and renal function in the normal conscious, chronically catheterized male rat[a]

	BP (mm Hg)	GFR (ml/min per 100 g body wt)	RPF	FF	RVR (mm Hg/(mL/min))	V (μL/min)	$U_{Na}V$ (μEq/min)	FE_{Na} (%)
Group 1								
Control	117 ± 3	0.73 ± 0.03	2.85 ± 0.25	0.27 ± 0.03	6.28 ± 0.67	15.8 ± 1.7	1.51 ± 0.30	0.38 ± 0.56
+ NAME	150 ± 6	0.60 ± 0.05	1.82 ± 0.20	0.35 ± 0.03	12.30 ± 0.97	53.9 ± 1.5	4.08 ± 1.46	1.32 ± 0.44
$P <$	0.001	0.02	0.01	0.05	0.005	0.05	0.05	0.05
Group 2								
Control	119 ± 3	0.92 ± 0.06	3.46 ± 0.22	0.27 ± 0.01	5.56 ± 0.28	18.2 ± 2.0	1.88 ± 0.40	0.40 ± 0.09
+ AII	130 ± 5[b]	0.92 ± 0.05[b]	2.97 ± 0.22[b]	0.32 ± 0.02	7.33 ± 0.61[b]	17.7 ± 5.1[b]	2.78 ± 0.77	0.59 ± 0.17
$P <$	0.05	NS	0.05	0.05	0.05	NS	NS	NS
Group 3								
Control	114 ± 4	0.82 ± 0.06	3.52 ± 0.54	0.25 ± 0.03	4.98 ± 0.39	20.0 ± 3.9	2.04 ± 0.51	0.47 ± 0.12
+ NAME + AII	153 ± 5[c]	0.47 ± 0.02[c]	1.06 ± 0.12[b,c]	0.46 ± 0.04[b,c]	21.93 ± 2.01[b,c]	63.8 ± 14.9[c]	4.90 ± 1.72	1.85 ± 0.63[c]
$P <$	0.001	0.005	0.005	0.001	0.005	0.05	NS	NS

[a] Group 1 ($N = 7$) received the NO synthesis inhibitor NAME (10 mg/kg iv). Group 2 ($N = 8$) received AII alone (5 ng/kg per minute iv). Group 3 ($N = 6$) received NAME (10 mg/kg iv) plus AII (5 ng/kg per minute iv). P values given in the table are the within-group differences by paired t test. Other statistical comparisons are by two-way ANOVA. All data are given as mean ± SE. NS, not significant. See text for definition of other abbreviations.

[b] Different versus Group 1 (+ NAME).

[c] Different versus Group 2 (+ AII).

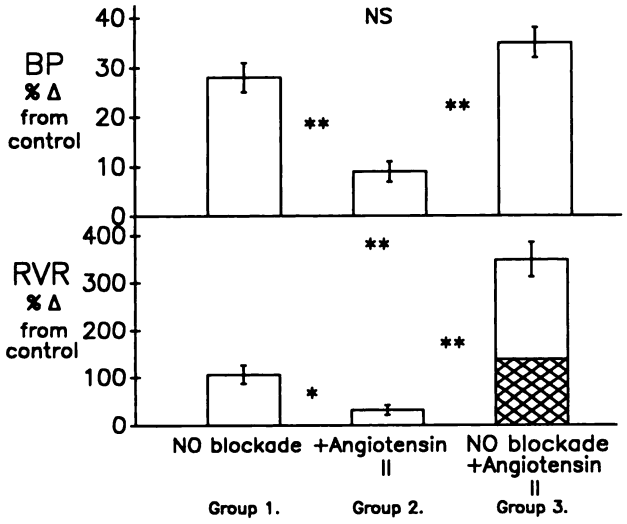

Figure 1. The percent change from control in BP (upper panel) and RVR (lower panel) in Group 1 rats receiving NO blockade alone (10 mg/kg of NAME iv), in Group 2 rats receiving an infusion of AII (5 ng/kg per minute iv) alone, and in Group 3 rats receiving combined NO blockade and AII infusion. The hatched column in percent change in RVR for Group 3 shows the arithmetic sum of the individual increases in RVR in Groups 1 and 2. NS, not significant. $P < 0.01$; ** $P < 0.001$.

blockade and an infusion of AII. In these rats, BP rose by a similar amount as in Group 1 but the RVR increase was much more profound, and as shown in Figure 1, RVR rose by approximately 350% versus 100% in Group 1. As also shown, the magnitude of the rise in RVR in Group 3 with combined NO blockade and AII infusion, exceeded by more than two times the arithmetic sum of the increases in RVR from Groups 1 and 2. Thus, acute NO blockade in a high AII state leads to a synergistic vasoconstriction that is selective to the kidney. This intense renal vasoconstriction led to marked reductions in RPF and GFR, despite an increase in FF (Table 1). A diuretic response persisted in Group 3 rats and the natriuretic response was numerically similar to that in Group 1 rats (which received NO blockade alone), although the increased variability in Group 3 prevented the increase in sodium excretion from reaching statistical significance.

DISCUSSION

Our earlier studies in the conscious rat show that a marked renal vasoconstriction is produced by acute NO blockade that does not involve the endogenous AII system (4). In this unstressed, conscious rat preparation, activity of the endogenous AII system is at the normal, unstimulated value and AII is not controlling RVR, although sodium excretion is tonically regulated by AII (1,6). A similar lack of AII involvement in NO blockade–induced renal vasoconstriction has been reported by Pucci and colleagues in the anesthetized rat (8). However, other workers have reported that AII blockade markedly attenuates the renal vasoconstrictor response to acute systemic NO blockade in the anesthetized rat and also in the "*in vitro*" juxtamedullary nephron preparation (2,3,9,). The AII dependence of NO blockade–induced renal vasoconstriction varies with the preparation, being much less pronounced in the awake rat than when the AII system is activated by acute surgery and/or volume depletion (5). These observations suggest that when endogenous AII levels are sufficiently high to control RVR, NO is important in maintaining renal perfusion.

In this study, we gave exogenous AII in a dose that produced a small direct vasoconstriction of the kidney. When this dose of AII was combined with concomitant NO blockade, a massive renal vasoconstriction resulted that caused severe impairments in RPF and GFR. The rise in RVR was so marked that it greatly exceeded the purely additive effect of each maneuver alone, suggesting a synergism between AII infusion and NO blockade. In preliminary studies by others, in the conscious dog, a similar amplification of AII-induced renal vasoconstriction by NO blockade has been reported (10). *In vitro* studies in isolated rabbit renal arterioles suggest that this interaction is confined to the afferent arteriole because the blockade of tonically produced NO enhances the vasoconstrictor response to administered AII of the afferent, but not efferent, arteriole (11). When intrarenal AII levels are chronically elevated, by either infusion or renal artery stenosis, tonically produced NO appears to be less important in the maintenance of renal perfusion (12,13). Perhaps other compensatory vasodilator systems are activated in response to chronic increases in the activity of intrarenal AII.

Locally produced NO acts directly to blunt the renal vasoconstrictor actions of AII by providing an opposing vasodilatory stimulus; this interaction is therefore presumably not specific but will occur, whatever the prevailing vasoconstrictor agent. For example, when norepinephrine is infused into the dog kidney in a mildly vasoconstrictor dose, concomitant NO inhibition has been shown to greatly exacerbate the renal hemodynamic impairment (14). In addition to functioning as a directly acting vasodilator, NO may also limit the activity of vasoconstrictors by acting directly at the receptor to reduce the duration of receptor-ligand interaction (15). NO blockade may also increase AII levels because some workers report that NO is inhibitory on renin release (16–18), although this is a controversial area (19–21).

Systemic NO blockade in the conscious rat causes a natriuresis and a diuresis that are probably secondary to the rise in BP (1), because the direct action of

NO blockade on sodium transport should be antinatriuretic (22). In contrast to the potentiated renal vasoconstrictor response to AII infusion and NO blockade, there was no amplification of the natriuretic/diuretic response seen with NO blockade alone. In addition to the pressure natriuretic effect, there are likely to be complex influences on sodium excretion, which include direct actions of AII on epithelial transport, removal of direct actions of NO on epithelial transport, and reductions in GFR and RPF. It is surprising, however, that, despite a large fall in the filtered load of sodium, combined AII infusion and NO blockade showed little tendency to reduce sodium excretion or urine flow.

In summary, these observations suggest that NO is very important in maintaining renal perfusion and glomerular filtration when AII is acutely elevated to a level sufficient to control renal hemodynamics. The dissociation between the renal and the systemic responses to NO blockade during AII infusion suggests that NO release is selectively raised within the renal vasculature as AII levels are elevated.

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